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CLINICAL INVESTIGATION

Metabolic clearance rate and production rate of calcitriol in uremia

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Metabolic clearance rate and production rate of calcitriol in uremia. We have previously demonstrated that while both normal humans and dogs tightly control serum calcitriol levels after 25(OH)D administration, anephric humans and 5/6 nephrectomized dogs significantly increase circulating $1,25(OH)_2D$ when supraphysiological concentrations of 25(OH)D are reached in serum. Plasma $1,25(OH)_2D$ level is determined not only by its rate of production but also by its rate of degradation. To further characterize the mechanisms involved in the responses to 25(OH)D therapy in normal circumstances and in chronic uremia, we measured metabolic clearance rate (MCR) and production rate (PR) of $1,25(OH)_2D$ in normal dogs and in dogs with moderate and severe renal failure, at normal and supraphysiological serum concentrations of 25(OH)D. Basal MCR in uremic dogs, either with moderate or with severe renal failure, did not differ significantly from normals (6.7 ± 0.7, 6.8 ± 0.4 and 6.8 ± 0.3 ml/min, respectively). Oral 25(OH)D administration for two weeks did not affect MCR either in normal animals or in both groups of uremic dogs. 25(OH)D treatment did not affect production rates in normal dogs and in animals with moderate renal failure (with normal basal values of $1,25(OH)_2D$), but significantly increased $1,25(OH)_2D$ production from 0.13 ± 0.01 to 0.25 ± 0.04 μg/day ($P < 0.05$) in dogs with severe renal insufficiency. These data suggest that it is the basal level of $1,25(OH)_2D$ which regulates the synthesis of $1,25(OH)_2D$ in response to 25(OH)D administration in normal and uremic animals.

$1,25(OH)_2D$ synthesis is strictly dependent upon an adequate level of vitamin D [1–3]. However, serum calcitriol levels are not affected by increased vitamin D uptake either in normal adults [4, 5] or intact chicks and rats [6]. A previous report from our laboratory confirmed these findings in normal humans after oral 25(OH)D administration and also demonstrated a similar pattern in normal dogs. However, 25(OH)D administration significantly increased serum $1,25(OH)_2D$ concentrations in anephric humans and 5/6 nephrectomized dogs [7]. Thus, the mechanisms operating to tightly control $1,25(OH)_2D$ concentrations after substrate challenge under normal circumstances appear to be absent or abnormal in chronic uremia. As serum $1,25(OH)_2D$ level is not only determined by its rate of production but also by its rate of degradation, we measured metabolic clearance rate (MCR) and production rate (PR) in normal and uremic animals under baseline conditions and after the administration of 25(OH)D.

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Methods

Seven normal mongrel dogs and 12 uremic dogs (5/6 nephrectomy) weighing 16 to 23 kg were studied. Four dogs with moderate renal failure had a GFR of 17.3 ± 2.4 ml/min and eight dogs with severe renal failure had a GFR of 9.6 ± 1.7 ml/min. A diet providing 1.6 g calcium and 1.5 g phosphorus per day was used to prevent hypercalcemia. Basal studies were performed three weeks after feeding the dogs the above described diet. Oral $25(OH)D_3$, at a dose of 100 μg every other day for two weeks, was administered to seven normal and eight uremic dogs (4 with moderate and 4 with severe renal failure). Blood samples were drawn before the metabolic clearance rate measurements to evaluate PTH, ionized calcium, phosphorus, creatinine, 25(OH)D and $1,25(OH)_2D$ levels. Serum ionized calcium was measured by an ion specific flow through electrode (Model SS20, Orion Research, Inc. Cambridge, Massachusetts, USA). Serum phosphorus was measured by autoanalyzer II. (Technicon Instruments, Tarrytown, New York, USA.) A highly sensitive animoterminal PTH radioimmunoassay was used to measure PTH levels in dogs [8]. 25(OH)D was measured with a radio receptor assay using sheep serum as the source of binding protein after purification of the dog 25(OH)D sample using a C18 Sep Pak column [9]. $1,25(OH)_2D$ was measured using the method developed by Reinhardt et al [10]. In the extraction procedure, the amount of 96:4 hexane:isopropanol was doubled to assure complete elution of 25(OH)D. Metabolic clearance rates and production rates were measured according to the method of Seeman et al [11] after a single dose of $[^3H]$-$1,25(OH)_2D$. 0.8 μCi of $1,25\text{-}(OH)_2[26,27\text{-}^3H]D_3$ (130 to 180 Ci/mmol, Amersham, Arlington Heights, Illinois, USA) in 1 ml polypropylene glycol were injected intravenously with rapid and repetitive rinsing by withdrawal and reinfusion of blood in the limb leg vein. Serial 5 ml blood samples were drawn at 0.5, 1, 2, 4, 6, 8, 10, 15, 20, 30, 45, 60, 90, 120, 240, 360, 480, 600 and 1440 minutes from a neck catheter. Total tritium in 1 ml plasma from the serial blood samples was measured in a Beckman Model LS 2800 beta counter (Beckman Instruments, Fullerton, California, USA). Counts per minute were converted to disintegrations per minute using an efficiency versus H number regression analysis. Average counting efficiency and counting error were 28.8% ± 2.2 and 3.6% ± 0.1, respectively.

Table 1. Relevant serum measurements before (basal) and after 25(OH)D treatment in dogs with normal renal function, moderate and severe renal failure

	Normal		Moderate		Severe	
	Basal	25(OH)D	Basal	25(OH)D	Basal	25(OH)D
Creatinine *mg/dl*	0.79 ± 0.05	0.77 ± 0.03	2.1 ± 0.2^{c}	2.0 ± 0.3^{c}	3.0 ± 0.5^{c}	3.1 ± 0.4^{c}
25(OH)D *ng/ml*	33.7 ± 3.1	152.4 ± 8.8^{f}	43.0 ± 7.2	106.8 ± 8.8^{b,f}	35.3 ± 5.3	176.3 ± 17.8$_{b}^{e}$
1,25(OH)$_2$D *pg/ml*	24.6 ± 1.4	25.0 ± 1.7	23.4 ± 1.0	24.9 ± 1.0	14.7 ± 1.1$_{c}^{c}$	28.4 ± 0.6^{e}
ICa *mg/dl*	5.1 ± 0.09	5.2 ± 0.05	5.3 ± 0.06	5.4 ± 0.03	5.4 ± 0.1	5.2 ± 0.35
P *mg/dl*	3.1 ± 0.3	2.9 ± 0.2	4.6 ± 1.2	4.6 ± 0.9	6.6 ± 1.2^{b}	8.5 ± 0.7$_{c}^{c}$
PTH *pg/ml*	19.4 ± 3.5	15.5 ± 2.9	50.0 ± 7.8^{a}	37.7 ± 4.6	105.0 ± 15.6$_{c}^{c}$	142.5 ± 28.5$_{c}^{c}$

a, b and c represent $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively, for statistical differences from normal (superscript) and from moderately uremic dogs (subscript).
e and f indicate $P < 0.01$ and $P < 0.001$ for statistical differences within experimental groups.

Serum pools were prepared for each time point for assessment of authentic [^{3}H]1,25(OH)$_2$D$_3$. Cold 1,25(OH)$_2$D$_3$ was added to each sample to quantitate recoveries. Samples were extracted using C18 and Silica Sep Pak according to Reinhardt et al [10] and the 1,25(OH)$_2$D fraction collected was dried under nitrogen, redissolved in 200 μl of 5% isopropanol in methylene chloride and further purified by using a HPLC Zorbax Sil column and methylene chloride:isopropanol (19:1) as the solvent system. The fraction coeluting with 1,25(OH)$_2$D was collected and counted. Average recovery after HPLC, efficiency for tritiated 1,25(OH)$_2$D counting and counting error were 80.7 ± 2.3; 47.4 ± 2.8 and 4.7 ± 0.17%, respectively. Percent of authentic 1,25(OH)$_2$D was calculated for each time point. Total tritium in serial serum samples for each dog was corrected for the percent of authentic 1,25(OH)$_2$D found in the pool. As 1α,25-dihydroxyvitamin D$_3$-26,23 lactone (1-alpha-lactone) coeluted with 1,25(OH)$_2$D$_3$ using the HPLC system described above [12], the contribution of 1-alpha-lactone to the tritiated 1,25(OH)$_2$D$_3$ fraction was checked by an additional HPLC purification using a Zorbax Sil column and 15% isopropanol in hexane at a flow rate of 1.2 ml/min. Retention time for 1,25(OH)$_2$D and 1-alpha-lactone were 7.5 and 13.3 minutes, respectively. No detectable amount of tritium eluted in the 1-alpha-lactone fraction at 2, 4, 6, 8, 10 and 24 hours after the bolus injection of tritiated 1,25(OH)$_2$D in any of the experimental circumstances studied. These results agree with previous findings that under physiological serum levels of 1,25(OH)$_2$D, 1-alpha-lactone appeared to be a minor product of 1,25(OH)$_2$D catabolism [13, 14]. Compartmental analysis of isotope disappearance from plasma was carried out using the program developed by the Statistical Analysis System Institute [15]. A three compartment model produced the best fit for the plasma ^{3}H 1,25(OH)$_2$D disappearance curve in 73.5% (25/34) of the dogs studied. A two compartmental model resulted in the best fit in the remainder. In all cases, parameters obtained from the best fit model were used to extrapolate to infinity. The metabolic clearance rate was calculated by dividing the administered dose by the time integral of the area under the curve extrapolated to infinity. Assuming steady state conditions, production rate was calculated as the product of the metabolic clearance rate and the plasma concentrations of 1,25(OH)$_2$D [16]. Paired *t*-test analysis was employed to quantitate statistical differences before and after treatment within experimental groups and analysis of variance was used to analyze the differences between groups.

Table 2. The effects of 25(OH)D administration on M.C.R. (ml/min) in normal dogs and in dogs with moderate and severe renal failure

	Normal	Moderate	Severe
Basal	6.8 ± 0.3 (7)	6.7 ± 0.7 (4)	6.1 ± 0.9 (4)
After 25(OH)D	5.8 ± 0.3 (7)	6.3 ± 0.2 (4)	6.0 ± 0.8 (4)

Results

Table 1 shows serum concentrations of creatinine, 25(OH)D, 1,25(OH)$_2$D, ionized calcium, phosphorus and iPTH in normal dogs and in dogs with severe and moderate renal failure before and after oral administration of 100 μg of 25(OH)D every other day for two weeks. (mean ± SEM, N = number of animals). 25(OH)D administration significantly increased serum 1,25 (OH)$_2$D levels in dogs with severe renal failure. This treatment did not result in enhanced concentrations of calcitriol in dogs with either normal renal function or moderate renal failure. Although in moderate uremia, serum 25(OH)D concentrations did not achieve the values observed in normal or severely uremic animals, previous observations from our laboratory [7] have demonstrated that this two- to threefold increase in circulating 25(OH)D was sufficient to significantly increase serum 1,25(OH)$_2$D levels in uremia. No significant differences between serum levels of ICa, P or iPTH occurred with 25(OH)D treatment either in normal or uremic animals, suggesting that these modulators of 1-alpha-hydroxylase activity were not determining the different responses observed.

Metabolic clearance rates and production rates were measured as described in **Methods**.

MCR values before and after 25(OH)D administration are shown in Table 2. Basal MCR measurements in normal animals were in close agreement with those reported by Eastell, [17] using a primed continuous infusion method in dogs of similar body weight. Basal MCR in moderate or severe renal failure did

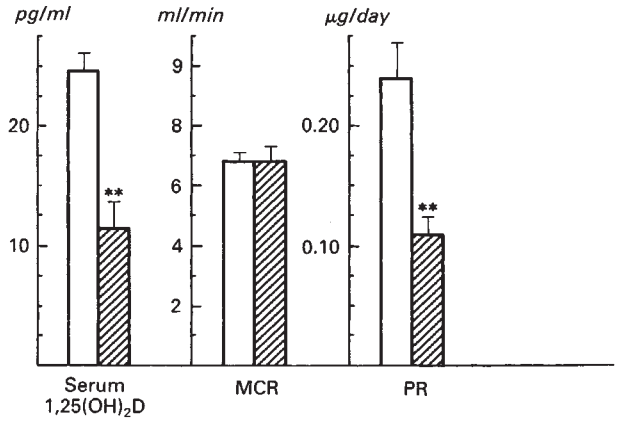

Fig. 1. *Serum levels, metabolic clearance rate and production rate of calcitriol in normal (□, N = 7) and severely uremic (▨), N = 8) dogs.* Mean (bar) ± SEM. $^{**}P < 0.01$.

not differ significantly from normals. Although oral 25(OH)D intake appeared to slightly decrease MCR of $1,25(OH)_2D$ in normal animals, analysis of variance showed no significant differences in the MCR of calcitriol after 25(OH)D therapy between experimental groups (Table 2).

$1,25(OH)_2D$ synthesis (PR) was significantly lower than normal in severely uremic animals. (Normals: 0.24 ± 0.02 μg/day, $N = 7$; uremics: 0.11 ± 0.01, $N = 8$; $P < 0.01$). This reduced production determined the significantly lower circulating levels of the hormone in severe uremia (Fig. 1).

Figure 2 summarizes the PR measurements before and after 25(OH)D treatment in dogs with normal renal function, moderate and severe renal failure.

Basal PR in moderate uremia did not differ from normal. 25(OH)D treatment did not affect significantly PR in normal animals or in dogs with moderate renal failure. In all four of the eight severely uremic dogs that received 25(OH)D, PR significantly increased. (Paired t-test analysis: $P < 0.05$).

Clearly, an increase in $1,25(OH)_2D$ production after 25(OH)D administration was observed only when basal serum $1,25(OH)_2D$ concentrations were significantly lower than normal. The increased PR after 25(OH)D administration in severe uremia did not differ significantly from the values observed for normal and moderately uremic dogs.

Discussion

Previous studies from our laboratory have demonstrated that serum calcitriol concentrations are not affected by supraphysiological 25(OH)D levels in normal adults and in normal dogs. On the contrary, 5/6 nephrectomized dogs and anephric humans significantly increased serum $1,25(OH)_2D$ after oral administration of 25(OH)D [7].

Circulating levels of calcitriol depend not only upon its rate of production but also upon its rate of degradation, thus in an attempt to clarify the mechanisms determining the diverse response described above, we measured MCR and PR of calcitriol in normal and uremic dogs.

MCR measurements for normal and severely uremic animals at physiological levels of 25(OH)D demonstrated that a reduction in renal mass did not affect significantly the catabolic rate of the active metabolite of vitamin D. In humans, no significant differences in calcitriol half life have been found between normal and uremic subjects [18]. These studies suggest that other tissues might be major sites for calcitriol inactivation as reported by Kumar and DeLuca [19].

In rats, however, there is controversy in the literature with regard to the effect of the reduction in renal mass in the MCR of calcitriol. While no changes in MCR of calcitriol were found by Taylor et al [20] after unilateral nephrectomy or by Hsu et al after acute tubular necrosis [21], Hsu et al reported a significant reduction in MCR in unilaterally nephrectomized rats [22]. Moreover, Hsu et al also found a reduction in MCR of calcitriol when a uremic state was created in rats with normal renal function [21].

In dogs with severe renal failure, serum $1,25(OH)_2D$ concentrations were significantly lower than in normal animals. Metabolic clearance rate was not affected by 25(OH)D administration, thus indicating that the increased circulating levels of $1,25(OH)_2D$ after treatment were determined by an enhanced synthesis of the hormone as shown by PR measurements. These results imply that changes in serum $1,25(OH)_2D$ either in normal dogs or in dogs with renal failure are controlled by regulation of calcitriol synthesis.

Although there is consistent evidence that serum $1,25(OH)_2D$ varies in direct proportion with GFR, [23–29), a wider dispersion of the data around the regression line is observed at higher GFR. Studies in patients with early renal failure have shown that serum levels of vitamin D metabolites vary from normal to clearly reduced values, suggesting that factor other than GFR contribute to determining serum $1,25(OH)_2D$ concentrations [29].

The complex regulation of 1α-hydroxylase by PTH [30–36], calcium [37–41], phosphorus [42, 43], and $1,25(OH)_2D$ [30, 31] itself has been extensively explored in vivo and in vitro. In patients with variable degree of renal failure no significant correlations between serum $1,25(OH)_2D$ and serum calcium or PTH were found, suggesting that the stimulatory effect on 1α-hydroxylase described for PTH in vitro cannot prevent the progressive decrease in serum calcitriol. However, multiple regression analysis showed a significant inverse correlation between $1,25(OH)_2D$ and serum phosphorus [27].

In agreement with these reports, our severely uremic dogs (serum iPTH and P levels were significantly higher than normal) had significantly impaired $1,25(OH)_2D$ synthesis but the potential to respond to supraphysiological 25(OH)D levels and normalize production. The stimulation of 1α-hydroxylase after 25(OH)D administration observed in severely uremic dogs and absent in normal animals cannot be attributed to changes in serum PTH, ionized calcium or phosphorus levels. Thus, to test the role of serum $1,25(OH)_2D$ concentration in the control of its own synthesis, we studied dogs with moderate renal insufficiency (early renal failure) with normal serum levels of $1,25(OH)_2D$.

Basal MCR of $1,25(OH)_2D$ was not significantly different from normal and remained unchanged after 25(OH)D therapy. Circulating $1,25(OH)_2D$ concentrations were not changed by increased serum 25(OH)D, suggesting that $1,25(OH)_2D$ at physiological levels regulates its own synthesis.

In summary, 1) Severe renal failure does not affect the metabolic clearance rate of calcitriol. 2) In normal animals, the

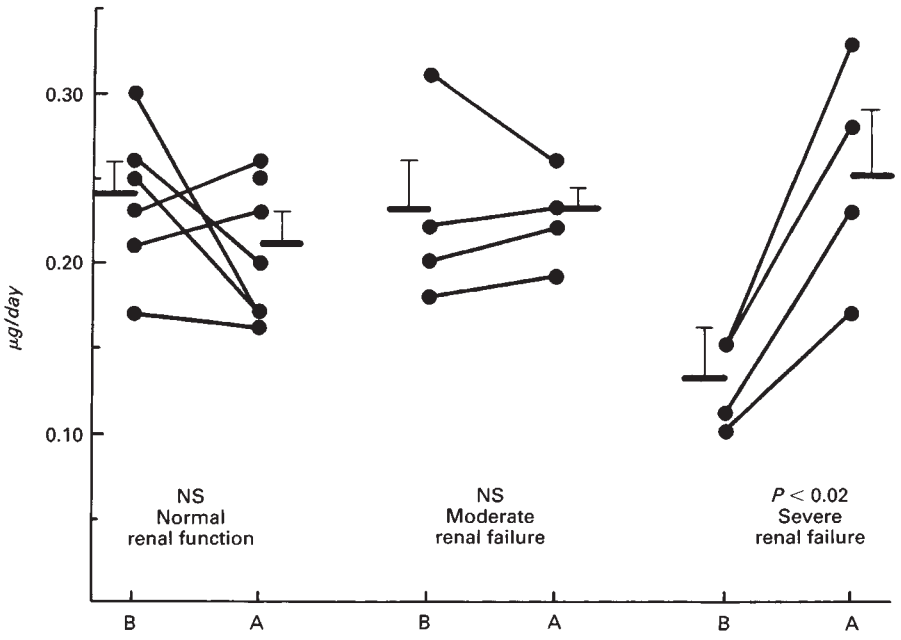

Fig. 2. *Production rates of calcitriol in dogs with normal renal function, moderate and severe renal failure before (B) and after (A) oral 25(OH)D administration (closed circles).* Mean (horizontal bars) ± SEM are plotted at both sides of individual values. Paired *t*-test and analysis of variance were used as indicated in **Methods**.

constant levels of $1,25(OH)_2D$ after 25(OH)D treatment are not explained by accelerated catabolism of calcitriol but rather by a regulated production of the hormone. 3) In moderate renal insufficiency, MCR and PR of calcitriol are not significantly different from normal values. 25(OH)D therapy does not affect serum levels, MCR or PR of $1,25(OH)_2D$. 4) In severe renal failure, an impaired $1,25(OH)_2D$ production leads to lower than normal serum concentrations of the hormone. 25(OH)D administration does not affect MCR but significantly enhances circulating $1,25(OH)_2D$ levels by increasing production rate.

In conclusion: Supraphysiological 25(OH)D levels stimulate 1α-hydroxylase activity only when serum $1,25(OH)_2D$ concentrations are below normal suggesting that, either in normal or uremic states, physiological serum levels of $1,25(OH)_2D$ regulate its own production.

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